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# Belowground herbivore tolerance involves delayed over compensatory root regrowth in maize

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**Short Title**: Root Regrowth Timing upon Herbivory

25 **ABSTRACT**

26 Plants can tolerate leaf-herbivore attack through metabolic reconfigurations that allow for the rapid
27 regrowth of lost leaves. Several studies indicate that root-attacked plants can re-allocate resources to
28 the above ground parts. However, the connection between tolerance and root regrowth remains
29 poorly understood. We investigated the timing and extent of root regrowth of tolerant and susceptible
30 maize lines *Zea mays* L. (Poaceae) attacked by the western corn rootworm *Diabrotica virgifera virgifera*
31 LeConte (Coleoptera: Chrysomelidae) in the laboratory and the field. Infested tolerant maize plants
32 produced more root biomass and even over compensated for the lost roots, while this effect was
33 absent in susceptible lines. Furthermore, the tolerant plants slowed growth of new roots in the
34 greenhouse and in the field 4-8 days after infestation, while susceptible plants slowed growth of new
35 roots only in the field and only after 12 days of infestation. The quick response of tolerant lines may
36 have enabled them to escape root attack by starving the herbivores and by saving resources for
37 regrowth after the attack had ceased. We conclude that both timing and the extent of regrowth may
38 determine plant tolerance to root herbivory.

39 **INTRODUCTION**

40 The capacity of crops to maintain high yields under adverse environmental conditions is of central
41 importance for sustainable farming. Under herbivore attack, yields can be protected through
42 resistance and tolerance mechanisms. Resistance refers to the capacity of plants to reduce herbivore
43 injury (Howe & Jander 2008), while tolerance refers to the capacity of plants to maintain productivity
44 upon sustained damage (Strauss & Agrawal 1999). One advantage of plant tolerance could be that it
45 would reduce selection pressure on the herbivores and thereby avoids the risk of counter adaptations
46 (Stowe et al. 2000). On the other hand, tolerance mechanisms *per se* can have a physiological cost to
47 the plant, as they would need to compensate for the herbivore- imposed loss of biomass (Pilson 2000;
48 Stinchcombe 2002; Fornoni et al. 2004 but see Mauricio et al. 1997; Agrawal et al. 1999; Boege et al.
49 2007). Plant tolerance to leaf-herbivore attack has been widely studied. Tolerance responses include

50 the activation of meristematic growth, which can be supported by an increase in photosynthesis
51 (Schultz et al. 2013; Strauss & Agrawal 1999). Photoassimilates can also be reallocated to non-attacked
52 tissues for future regrowth (Babst et al. 2005; Babst et al. 2008; Gómez et al. 2010; Hanik et al. 2010;
53 Henkes et al. 2008; Holland, Cheng & Crossley 1996; Schwachtje et al. 2006). In many cases however,
54 leaf attack by small herbivores triggers a decrease in photosynthesis and leads to a depletion of
55 photoassimilates (Machado et al. 2013). Grazing by larger browsers, on the other hand, can lead to
56 overcompensatory growth, in which case plants accumulate more biomass than without herbivory
57 (Paige & Whitham, 1996). The regular cutting of grasslands for maximal hay production can be seen as
58 an extreme grazing regime that takes advantage of the plant's capacity to tolerate tissue removal
59 (Hawkes & Sullivan 2001; Borer et al. 2014).

60 Unlike leaves, relatively little is known about the mechanisms that may enable plants to tolerate root
61 feeding insects. Root herbivores are among the most important pests in a number of crops. Due to
62 their secluded life style, they often remain difficult to combat with traditional means and have been
63 studied in much less detail than their leaf counterparts (Hunter 2001; Erb et al. 2013). Field studies of
64 maize and its most devastating root pest, the western corn rootworm *Diabrotica virgifera virgifera*,
65 have documented that certain maize varieties are able to maintain high productivity after being injured
66 by *D. virgifera* (Prischmann et al. 2007), pointing to the presence of effective tolerance mechanisms. A
67 $^{13}C$ labelling approach revealed marginally increased allocation of photosynthates to the above ground
68 parts of *D. virgifera* attacked maize (Xue et al. 2012). Using $^{11}C$ as a tracer revealed that attacked maize
69 seedlings allocated significantly more carbon to the stems, an effect which was associated with an
70 increase in stem thickness and increased crown root growth (Robert et al. 2014). It is therefore
71 conceivable that maize tolerates *D. virgifera* attack through carbon reallocation followed by
72 compensatory growth. Furthermore, a study on *Centaurea maculosa* showed that the plant increases
73 nitrogen (N) allocation to the shoot when attacked by the root feeder *Agapeta zoegana* (Newingham,
74 Callaway & BassiriRad 2007), which indicates that N reallocation may also help plants to cope with root
75 attack.

For root herbivore tolerance, the timing of regrowth may be an important parameter. *D. virgifera* for instance has a strong preference for young crown roots (Robert et al. 2012), and often prunes them directly after they penetrate the soil surface. Hence immediate regrowth following attack may backfire on the plant by improving *D. virgifera* larval survival and vigour. A desirable regrowth program could instead involve a delay that saves the resources for root reestablishment until the larvae have died or pupated. To date, little is known about the importance of regrowth timing for root herbivore tolerance.

In this study, we investigated the regrowth patterns of susceptible and tolerant maize lines following *D. virgifera* attack in the field and the glasshouse. Using segregating genetic populations that were enriched in tolerance or susceptibility alleles, we tested 3 phenological hypotheses regarding the potential mechanisms of tolerance: First, we tested whether tolerant maize lines compensate for root loss by regrowing more strongly below ground. Second, we tested whether the differences in regrowth are associated with morphological changes in the stems as potential carbohydrate storage organs. Third, we tested whether there are differences in the timing of regrowth between tolerant and susceptible lines. Together, our experiments paint a detailed picture of the phenotypical traits that are involved in root herbivore tolerance and reveal that both timing and extent of regrowth are associated with the maintenance of crop productivity under root herbivore attack.

## MATERIAL AND METHODS

*Plants and insects*. The CRW17 maize population was used. The intent of the CRW17 population was to conduct a recurrent selection maize breeding program by selecting the ears from least damaged lines, bulk pollinating nursery rows (Ball 1969) of the selected ears, and evaluating again in a new cycle of selection as was done for CRW3 by (Hibbard et al. 2007). Development of this population began in 2007 by using 17 of the lines least damaged by *Diabrotica virgifera* (based on 15 years of evaluating thousands of maize lines) in a diallel scheme. The 17 lines ("founders") originated from several germplasm groups, most of which had more than one representative. Parental materials of the diallel included selections derived from the CRW3 population (Hibbard et al. 2007), selections derived from

Germplasm Enhancement of Maize (GEM) materials (Salhuana, W., and L. Pollak. 2006), and from BS19 and BS20 (Russell et al. 1976; Ball 1969). A balanced bulk set of seed from the diallel cross was planted in 2008 for initial recombination. Delayed plantings of this bulk insured that the variable flowering crosses were combined. A second balanced bulk seed set was created and recombined a second time in 2009. Finally, 300 selfed ears from the 2009-2010 winter nursery were selected as CRW17(C0). Nine kernels from each ear were planted in 1.5 m plots in two locations near Columbia in Missouri as well as one location near Brookings in South Dakota in 2010 (Hibbard *et al.*, unpublished data). Four roots from each plot were considered as one replication and each evaluated for plant injury (Oleson et al. 2005) as well as root regrowth and root size (Rogers et al. 1975). Plant injury is a linear rating scale and uses the following criteria: 0 = no root injury, 1 = one node of roots eaten, 2 = two nodes eaten, 3 = three nodes eaten. Root size and compensatory root growth were evaluated using 1 to 6 rating scales (1 = largest, 6 = smallest (Rogers et al. 1977)). Ears chosen for the current work were made with selections based upon consistent regrowth ratings. Three tolerant lines (significant regrowth after herbivory) CRW17-096, CRW17-101 and CRW209 as well as two susceptible lines (no regrowth after herbivory) CRW17-057 and CRW17-069 were used.

*Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) eggs were kindly provided by the USDA-ARS Columbia (MO, USA) and USDA-ARS-NACRL Brookings (SD, USA). Eggs were maintained in their oviposition dishes and stored in a growth chamber at 25$^{o}$ C and 60% RH until needed. For greenhouse experiments eggs were allowed to hatch and larvae reared on freshly germinated maize seedlings until use. Hatching of the eggs in the field was determined by keeping eggs in the lab and larval development was traced using soil temperatures as described in (Jackson & Elliott 1988; Hibbard et al. 2008).

*Greenhouse experiment*.

Maize seeds were sown in 1 L plastic pots (Pöppelmann, Lohne, Germany) containing a layer of moist washed sand (0-4 mm grain size, Raiffeisen, Germany) covered with 2 cm of commercial soil

126 (Tonsubstrat, Geeste, Germany; Aussaaterde, Ricoter, Aarberg, Switzerland). Seedlings were grown in
127 a greenhouse (23 ± 12 °C, 60% relative humidity, 16: 8 h L/D). Fertilizer (Ferty 3, Ferty®, Regenstauf,
128 Germany) was added twice a week after seed germination. Three week old plants were infested with
129 six second instar larvae for eight days. The infestation was performed by making two 5 cm deep and
130 0.5 cm diameter holes in the soil, at 2 cm distance from the maize stem each, in which the larvae were
131 placed. Such larval density allows injury levels relatively comparable to field injury and is commonly
132 used (Robert et al., 2012). Control plants remained uninfested. After this period, all roots were washed
133 with tap water and all larvae were manually removed from infested roots. Maize plants were then
134 replanted in soil. Stem circumference was measured at day 0 (day of infestation), day 8 and day 16
135 using a flexible ruler. The number of emerging crown roots was counted every four days post
136 infestation. Crown roots arise from the stem and were visible above the surface without disturbing the
137 plants. The number of emerging crown roots was expressed in percentage of newly emerged roots
138 compared to the average emerging crown from their respective controls.

139 *Field experiment*.

140 The field experiment was conducted in 2012 at the Bradford Research Extension Center (Columbia,
141 MO, USA). Maize plants (two seeds per plot) were sown on May 15$^{th}$ with 43-cm spacing between
142 plants and 76-cm spacing between rows. Plants of the variety Pioneer 33M16 were used as buffer
143 plants. Two rows of buffer plants surrounded each experimental plant to avoid herbivore movement
144 from infested to control plots and prevent edge effects. Two weeks after sowing, seedlings were
145 transplanted where no plant emerged. All experimental plots consisted of one plant. Plants from the
146 different maize lines were randomly placed in the field (n=48 per line). Two weeks later (May 30$^{th}$),
147 half of the plots (n=24) were infested with 600 *D. virgifera* eggs, which correspond to natural
148 infestation density (Hibbard *et al*., 2004; Robert et *al*., 2012). Control plants (n=24) were left
149 uninfested. Eggs from the same batch kept in the greenhouse hatched on June 11$^{th}$ (day 0). Stem
150 circumference, and crown root emergence was evaluated every four days after egg hatching. Crown
151 root emergence from infested plants was expressed as the percentage of newly emerged roots

compared to the average of their respective controls. Injury inflicted by *D. virgifera* was evaluated two weeks after larval hatching by harvesting half of the infested and half of the control plants (n=12 each) and using Oleson's scale (Fuller et al. 1997; Oleson et al. 2005). Root regrowth was recorded four weeks after larval hatching by harvesting the remaining plants (n=12 controls and n=12 infested plants) and attributing a score for regrowth on a scale from 0 to 6 (0= no regrowth; 6= much regrowth). Larval development was estimated using the model developed by Elliott *et al.* (1990). First harvest fell within the feeding phase of the larvae, while the second harvest fell within the pupation phase. Roots were then dried in a glasshouse with the cooling system turned off for 2 weeks prior to measuring their dry mass.

*Statistical analyses*

All statistical analyses were performed using R software (http://www.R-project.org/). Data were analyzed using a Wald test on a Linear Mixed Model (LMM; function 'lmer', package 'lme4' (Bates et al. 2014)) in which the phenotype and the treatment were considered as fixed factors, and the maize line as a random factor. Root damage and percentage of emerging crown roots were square root-transformed for a better model fit. Relevant pairwise comparisons of least squares means (LSMeans) were performed using the function 'lsmeans' (package 'lsmeans' (Lenth & Hervé 2015)) and the Tukey correction for P values. All LMM results can be found in table 1-7.

**RESULTS**

*Plant tolerance phenotype*. No difference was found in the root regrowth abilities of the susceptible lines. Similarly, no difference was found in the root regrowth abilities of the tolerant lines. Tolerant and susceptible lines suffered similar injury by the root herbivore *D. virgifera* in the field (Phenotype: $p>0.05$, Infestation: $p<0.001$, Interaction: $p>0.05$), suggesting no difference in resistance of the different lines (Figure 1). Susceptible and tolerant lines displayed similar root system biomass after two weeks of larvae feeding (Figure 2a). Yet, only tolerant plants regrew significantly more biomass

(Phenotype: $p>0.05$, Infestation: $p<0.01$, Interaction: $p>0.05$; Figure 2b) and roots (Phenotype: $p>0.05$, Infestation: $p=0.02$, Interaction: $p>0.05$; Figure 2c) four weeks after infestation. At the end of the experiment (four weeks feeding by the root herbivore), the root systems from infested tolerant plants were almost twice as large (volume and biomass) as the controls (Figure 2b and c).

*Stem circumference*. Susceptible lines did not grow larger stems upon belowground infestation by *D. virgifera* in the field (Figure 3a) or in the greenhouse (Figure 3b) at any of the measured time points. Tolerant lines displayed significantly larger stems upon infestation in the field at day 12 after larval hatching (Phenotype: $p>0.05$, Infestation: $p>0.05$, Interaction: $p=0.032$; Figure 3c) than control plants. However, it should be noted that this difference is driven by one of the tolerant plant lines only (CRW17C0-101) and that the effect is absent in all lines in the greenhouse (Figure 3d).

*Crown root emergence*. In the field, both susceptible and tolerant lines reduced the production of crown roots after *D. virgifera* infestation. Susceptible lines grew less crown roots after 12 days of belowground herbivory than their respective uninfested controls (Phenotype: $p=0.045$, Infestation: $p=0.02$, Interaction: $p=0.035$; Figure 4a). Infested tolerant plants reduced their production of new crown roots significantly after only eight days of herbivory (Phenotype: $p>0.05$, Infestation: $p=0.011$, Interaction: $p>0.05$; Figure 4c), but regrew some by day 12, after two weeks of infestation.

In the greenhouse, the same pattern was observed with a reduction in crown root emergence and regrowth occurring in tolerant lines (significant at day 4 after herbivory; Phenotype: $p>0.05$, Infestation: $p=0.004$, Interaction: $p>0.05$). Yet, no reduction was observed in susceptible lines before regrowth of new roots (Phenotype: $p>0.05$, Infestation: $p=0.005$, Interaction: $p>0.05$; Figure 4d).

## DISCUSSION

Our experiments reveal that both the extent and timing of regrowth may function in a synergistic manner to improve plant success under root herbivore attack.

200 Although both tolerant and susceptible maize lines were injured to the same extent by *D. virgifera*,
201 tolerant lines slowed down the growth of emerging roots early (4-8 days after attack) and regrew
202 significantly more roots four weeks after attack, while susceptible lines slowed down the emergence
203 of new roots only 12 days after infestation, only in the field, and did not overcompensate after four
204 weeks attack. In terms of biomass, the tolerant population produced more biomass when attacked by
205 *D. virgifera* than controls. This form of overcompensation has been observed in grazed plants before,
206 and it was suggested that grazing may in fact increase rather than decrease plant performance (Dyer
207 1975; McNaughton 1979; Hilbert et al. 1981; Paige & Whitham 1987). In the case of roots, it can be
208 expected that overcompensating maize plants maintain or even enhance their capacity to take up
209 water and nutrients from the soil later in the season. In fact, root regrowth after western corn
210 rootworm larval feeding positively affected yield when soil moisture is low, but negatively affected
211 yield when soil moisture was adequate (Gray & Steffey 1998). Therefore, and although the impact of
212 similar injury levels on tolerant and susceptible plant yield remains to be tested, it is tempting to
213 speculate that maintaining a large root system is an advantage late in the season upon *D. virgifera*
214 attack despite their high metabolic investment into root growth.

215 Apart from biomass over-accumulation, the tolerant maize lines displayed a significant shift in the
216 timing of root regrowth. While the susceptible population maintained its root system over 8 days of
217 herbivory and reduced its regrowth on day 12 in the field, the tolerant population reduced its regrowth
218 already after 8 days of infestation, and bounced back to control levels at day 12. A similar pattern was
219 observed under more controlled conditions in the greenhouse, albeit with a slightly earlier response
220 due to direct infestation with L2 larvae compared to starting with hatching eggs in the field. Although
221 the physiological reasons for these differences in regrowth timing remain to be determined, we
222 propose that a delayed regrowth may be advantageous for several reasons. First, an initial reduction
223 in below ground growth may starve the *D. virgifera* larvae, which may increase their mortality and
224 potentially their susceptibility to natural enemies. Second, the delayed burst of regrowth may co-occur
225 with the onset of larval pupation and the end of the feeding phase, thereby increasing the sustainability

of the newly produced roots. It is therefore possible that the delayed regrowth is partially responsible for the stronger over accumulation of root biomass in these lines. However, more detailed experiments are required to link the extent and timing of delayed root regrowth, including tighter experimental control of the timing of larval feeding. Nevertheless, it is tempting to speculate that the tolerant plants may be able to sense and anticipate the development of *D. virgifera* and thereby time their metabolic investment into regrowth.

In an earlier study, we observed that a maize hybrid line attacked by *D. virgifera* showed an increase in stem diameter, which was followed by increased crown root regrowth from the same organ (Robert et al. 2014). In the current experiments, however, we could not detect any effect on stem diameter. Only the stems from the tolerant line CRW17-101 were thicker upon *D. virgifera* attack from the day 0 onwards. This effect remains inconclusive as (i) infested plants possess thicker stems at day 0 in the field (although day 0 in the field is an approximation) and (ii) this difference was not found in the greenhouse. Several factors may account for the absence of a clear stem phenotype in the current experiments. First, it is possible that stem thickening is genotype specific, and that none of the lines used in the present study display this behaviour. Second, the stem phenotype may be visible only in younger plants, and not in the older plants used in the current experiments. As stems elongate and the different nodes move up from the stem base, it becomes more difficult to assess stem thickness as stems become somewhat irregular. From the current experiments, it seems that stem thickening is not a reliable marker of maize tolerance to root herbivory.

In conclusion, our study reveals that tolerant maize plants respond vigorously to root attack by the western corn rootworm with delayed over compensatory root growth. Although it remains to be determined whether other plant families display similar growth patterns, our results demonstrate how timing and vigour may act together to help plants endure herbivore attack. Given the limited availability of *D. virgifera* resistant maize germplasm, breeding for tolerance may be a promising alternative to reduce the negative impact of this root feeder. The observed growth phenotypes may

be useful markers to identify potentially tolerant germplasm in the greenhouse without the need to conduct laborious yield assessments.

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**FIGURE LEGENDS.**

Figure 1. Susceptible and tolerant lines were equally injured by the root herbivore *Diabrotica virgifera* in the field after two weeks of herbivory.

Figure 2. Root system mass and growth upon herbivory by *Diabrotica virgifera* in the field. Herbivory decreased the root dry biomass of both susceptible and tolerant phenotypes two weeks after larval hatching (A). Tolerant plants regrew higher biomass (B) and root volume (C) four weeks after larval hatching. Stars indicate significant differences. Ph: Phenotype; Inf: Infestation.

Figure 3. Stem circumference upon root herbivory by *Diabrotica virgifera* in both susceptible (A, C) and tolerant lines (B, D) in the field (A, B) and in the greenhouse (C, D). Stars indicate significant differences.

Figure 4. Emerging crown roots upon root herbivory by *Diabrotica virgifera* in both susceptible (A, C) and tolerant lines (B, D) in the field (A, B) and in the greenhouse (C, D). The number of emerging crown roots in each line after infestation is expressed as a percentage of emerging crown roots in plants of the same line grown as uninfested controls. Stars indicate significant differences.

**TABLES**

Table 1: MIXED MODEL table for root injury inflicted by *Diabrotica virgifera*. Values indicated in bold are significant. Ph: Phenotype (tolerant or susceptible); Inf: Infestation (control or infested); Ph * Inf: interaction phenotype-infestation.

| Factor: | Chisq | Df | pr(>Chisq) |
|---|---|---|---|
| Ph: | 0.035 | 1 | 0.852 |
| Inf: | **117.162** | **1** | **<2e-16** |
| Ph * Inf: | 0.020 | 1 | 0.881 |

Table 2: MIXED MODEL table for root dry weight after two weeks infestation by *Diabrotica virgifera*. Values indicated in bold are significant. Ph: Phenotype (tolerant or susceptible); Inf: Infestation (control or infested); Ph * Inf: interaction phenotype-infestation.

| Factor: | Chisq | Df | pr(>Chisq) |
|---|---|---|---|
| Ph: | 0.712 | 1 | 0.399 |
| Inf: | 3.012 | 1 | 0.083 |
| Ph * Inf: | 0.530 | 1 | 0.467 |

Table 3: MIXED MODEL table for root dry weight after four weeks infestation by *Diabrotica virgifera*. Values indicated in bold are significant. Ph: Phenotype (tolerant or susceptible); Inf: Infestation (control or infested); Ph * Inf: interaction phenotype-infestation.

| Factor: | Chisq | Df | pr(>Chisq) |
|---|---|---|---|
| Ph: | 0.274 | 1 | 0.601 |
| Inf: | **9.507** | **1** | **0.002** |
| Ph * Inf: | 1.851 | 1 | 0.174 |

Table 4: MIXED MODEL table for root regrowth after four weeks infestation by *Diabrotica virgifera*. Values indicated in bold are significant. Ph: Phenotype (tolerant or susceptible); Inf: Infestation (control or infested); Ph * Inf: interaction phenotype-infestation.

| Factor: | Chisq | Df | pr(>Chisq) |
|---|---|---|---|
| Ph: | 0.271 | 1 | 0.603 |
| Inf: | **5.258** | **1** | **0.022** |
| Ph * Inf: | 0.683 | 1 | 0.408 |

Table 5: MIXED MODEL table for plant stem circumference in the field. Values indicated in bold are significant. Ph: Phenotype (tolerant or susceptible); Inf: Infestation (control or infested); Ph * Inf: interaction phenotype-infestation.

| | Factor: | Chisq | Df | pr(>Chisq) |
|---|---|---|---|---|
| Field_Day 1 | Ph: | 0.268 | 1 | 0.605 |
| | Inf: | 3.444 | 1 | 0.063 |
| | Ph * Inf: | 0.261 | 1 | 0.609 |

| | | | | |
|---|---|---|---|---|
| Field_Day 5 | Ph: | 0.147 | 1 | 0.702 |
| | Inf: | 1.068 | 1 | 0.301 |
| | Ph * Inf: | 0.911 | 1 | 0.340 |
| Field_Day 9 | Ph: | 0.135 | 1 | 0.713 |
| | Inf: | 0.218 | 1 | 0.641 |
| | Ph * Inf: | 2.933 | 1 | 0.087 |
| Field_Day 12 | Ph: | 0.004 | 1 | 0.497 |
| | Inf: | 0.994 | 1 | 0.319 |
| | Ph * Inf: | **4.613** | **1** | **0.032** |
| Field_Day 16 | Ph: | 0.002 | 1 | 0.961 |
| | Inf: | 0.022 | 1 | 0.883 |
| | Ph * Inf: | 1.876 | 1 | 0.171 |

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Table 6: MIXED MODEL table for plant stem circumference in the greenhouse. Values indicated in bold are significant. Ph: Phenotype (tolerant or susceptible); Inf: Infestation (control or infested); Ph * Inf: interaction phenotype-infestation.

| | Factor: | Chisq | Df | pr(>Chisq) |
|---|---|---|---|---|
| Greenhouse_Day 0 | Ph: | 0.370 | 1 | 0.543 |
| | Inf: | 1.073 | 1 | 0.300 |
| | Ph * Inf: | 0.058 | 1 | 0.809 |
| Greenhouse_Day 8 | Ph: | 0.809 | 1 | 0.368 |
| | Inf: | 1.732 | 1 | 0.188 |
| | Ph * Inf: | 0.000 | 1 | 0.991 |
| Greenhouse_Day 16 | Ph: | 1.078 | 1 | 0.299 |
| | Inf: | 0.480 | 1 | 0.489 |
| | Ph * Inf: | 0.121 | 1 | 0.728 |

Table 7: MIXED MODEL table for the number of emerging crown roots in the field. Values indicated in bold are significant. Ph: Phenotype (tolerant or susceptible); Inf: Infestation (control or infested); Ph * Inf: interaction phenotype-infestation.

| | Factor: | Chisq | Df | pr(>Chisq) |
|---|---|---|---|---|
| Field_Day 0 | Ph: | 0.084 | 1 | 0.772 |
| | Inf: | 0.305 | 1 | 0.581 |
| | Ph * Inf: | 1.430 | 1 | 0.232 |
| Field_Day 4 | Ph: | 0.522 | 1 | 0.470 |
| | Inf: | 0.010 | 1 | 0.922 |
| | Ph * Inf: | 0.036 | 1 | 0.851 |
| Field_Day 8 | Ph: | 0.457 | 1 | 0.499 |
| | Inf: | **6.396** | **1** | **0.011** |
| | Ph * Inf: | 1.430 | 1 | 0.232 |
| Field_Day 12 | Ph: | **4.020** | **1** | **0.045** |
| | Inf: | 1.677 | 1 | 0.195 |
| | Ph * Inf: | **4.478** | **1** | **0.034** |

| Pairwise comparisons d8 | Estimate | SE | df | t ratio | p value |
|---|---|---|---|---|---|
| Phenotype = Susceptible Ctl - Herb | 1.640 | 1.356 | 214.230 | 1.210 | 0.228 |
| Phenotype = Tolerant Ctl - Herb | **2.564** | **1.125** | **214.610** | **2.280** | **0.024** |

| Pairwise comparisons d12 | Estimate | SE | df | t ratio | p value |
|---|---|---|---|---|---|
| Phenotype = Susceptible Ctl - Herb | **2.928** | **1.193** | **214.230** | **2.455** | **0.015** |
| Phenotype = Tolerant Ctl - Herb | -0.349 | 0.989 | 214.610 | -0.353 | 0.724 |

Table 8: MIXED MODEL table for the number of emerging crown roots in the greenhouse. Values indicated in bold are significant. Ph: Phenotype (tolerant or susceptible); Inf: Infestation (control or infested); Ph * Inf: interaction phenotype-infestation.

| | Factor: | Chisq | Df | pr(>Chisq) |
|---|---|---|---|---|
| Greenhouse_Day 4 | Ph: | 1.905 | 1.000 | 0.168 |
| | Inf: | **8.153** | **1.000** | **0.004** |
| | Ph * Inf: | 1.876 | 1.000 | 0.171 |
| Greenhouse_Day 8 | Ph: | 0.140 | 1 | 0.709 |
| | Inf: | 0.227 | 1 | 0.634 |
| | Ph * Inf: | 2.148 | 1 | 0.143 |
| Greenhouse_Day 12 | Ph: | 0.010 | 1 | 0.919 |
| | Inf: | 1.088 | 1 | 0.297 |
| | Ph * Inf: | 0.310 | 1 | 0.577 |
| Greenhouse_Day 16 | Ph: | 0.286 | 1 | 0.593 |
| | Inf: | **7.842** | **1** | **0.005** |
| | Ph * Inf: | 0.091 | 1 | 0.763 |

| Pairwise comparisons d4 | Estimate | SE | df | t ratio | p value |
|---|---|---|---|---|---|
| Phenotype = Susceptible Ctl - Herb | 1.949 | 2.305 | 68.620 | 0.846 | 0.401 |
| Phenotype = Tolerant Ctl - Herb | **6.128** | **2.009** | **68.090** | **3.050** | **0.003** |

| Pairwise comparisons d16 | Estimate | SE | df | t ratio | p value |
|---|---|---|---|---|---|
| Phenotype = Susceptible Ctl - Herb | **-3.236** | **1.601** | **70.610** | **-2.210** | **0.047** |
| Phenotype = Tolerant Ctl - Herb | -2.610 | 1.339 | 70.570 | -1.949 | 0.055 |